Supporting Information

Exploring the Feasibility of a Microchip Laser Ablation Method for the Preparation of Biopolymer-Stabilized Gold Nanoparticles: Case Studies with Gelatin and Collagen

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Instrumentations and Chemicals

Ultrapure water (18.2 $\Omega \cdot cm^{-1}$) was generated by an Organo Puric-ω water purification system. Centrifugal ultrafiltration was performed using a KUBOTA 7780II high speed refrigerated centrifuge attached with a RS-2504GS swing rotor. Freeze drying was performed using a Freeze dryer FDU-2200, Eyela (Tokyo, Japan). Transmission electron microscopy (TEM) images were recorded with a JEOL JEM-2100 electron microscope at an accelerating voltage of 200 kV using a Holey carbon support films coated Cu microgrid (EM Japan, U1003). The TEM grid was applied hydrophilic treatment in a glow discharge irradiation chamber before use. The obtained TEM images were analyzed using Image-J software, and the expressed mean diameter and standard deviation are based on the average of 300 particles. Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) was performed on a Shimadzu ICPS-8100 emission spectrometer. Circular dichroism (CD) spectroscopy was performed on a JASCO J-725 circular dichroism spectrometer. Dynamic Light Scattering (DLS) measurements were performed by Zetasizer (Nanoseries) Nano-ZS under 25 ˚C.

Three sets of the sample was prepared and the average value with standard deviation was calculated. Viscosity measurement was obtained by compact rheometer MCR 302 (Anton Paar, Modular) under 25 ^{0}C. Three sets of the sample was prepared for reproducibility purposes. Homogenization was performed using a Violamo VH-10 homogenizer equipped with a S10N-10G shaft generator (ø =10 mm, L = 115 mm).

The gold rod (> 99.99% purity, φ 5 mm × 15 mm) was purchased from Tanaka kikinzoku Kogyo K. K. The Gold rod was cleaned by ultrasonication in acetone for 5 min and rinsed with deionized water before use.

Unless otherwise noted, all reagents purchased from commercial suppliers were used without further purification.

Sodium chloride (NaCl) and Tris-HCl ($H_2NC(CH_2OH)_3$ · HCl) were purchased from FUJIFILM Wako Pure Chemical Corporation.

Phosphate-buffered saline powder (PBS) was purchased from Sigma–Aldrich Japan Inc.

Acetic acid (CH_3CO_2H) was purchased from KISHIDA CHEMICAL Co., Ltd.

Collagen pellets were obtained from Nippon Ham Foods Ltd. Gelatin powder was obtained from Osaka KISHIDA CHEMICAL Co., Ltd.

Dialysis membranes (MWCO, 15 kD) were purchased from Spectrum Laboratories, Inc.

Schematic and Description of Microchip Laser Apparatus

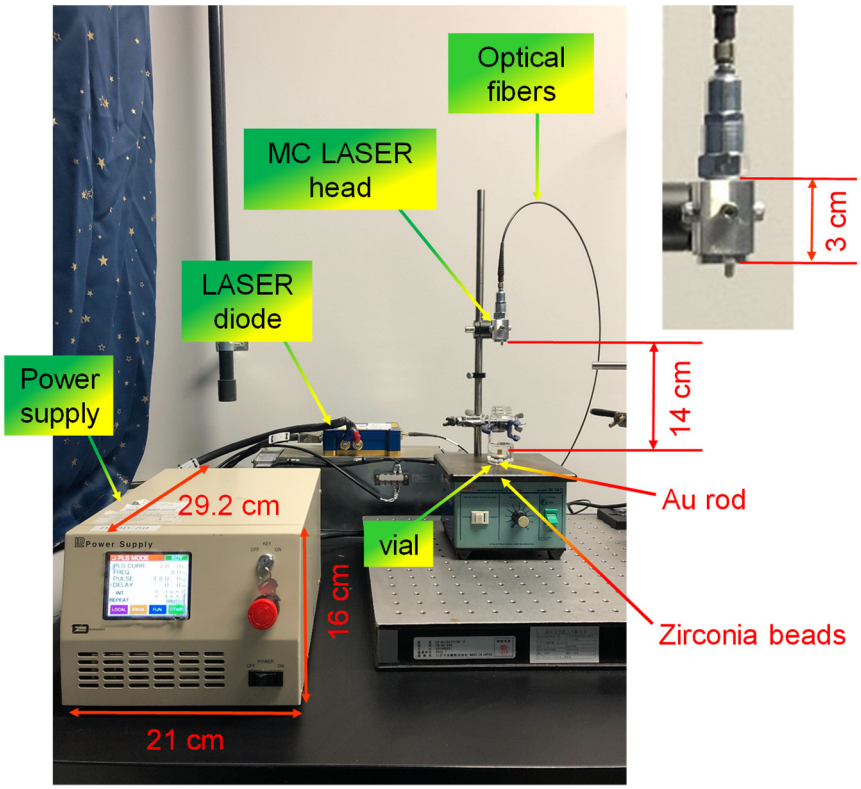


Figure S1. Photographs of the experimental setup used for microchip laser ablation.

The laser setup consists of three units; a PLSU 150 pulse laser power supply (UNITAC Co., LTD), a MWPN15 Nd:YAG/Cr^{4+}:YAG laser diode (UNITAC Co., LTD), and a microchip laser head equipped with a lens with a focal length of 250 mm. The microchip laser head was connected with the laser diode by an optical fiber with the length of 1 m (JENOPTIK AG). Laser irradiation was performed with repeated mode with a spot size of approximately 1.5–2.0 mm. Consistent laser parameters were applied across all experiments: wavelength, 1064 nm; pulse energy, 1.8 mJ; pulse duration, 900 ps; average laser power, 180 mW; repetition rate, 100 Hz.

TEM Characterization and Size Distribution of Gelatin-Stabilized Au NPs

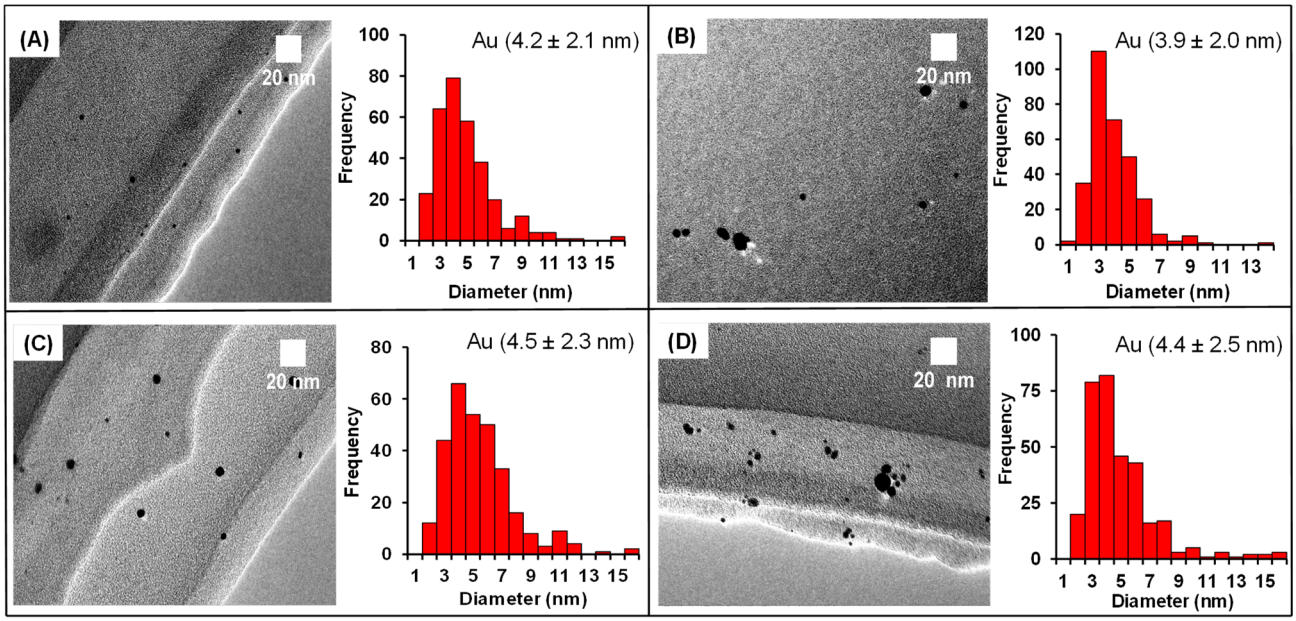


Figure S2. TEM images and size distribution histograms of Au:Gel nanoparticles at varying gelatin loadings: 0.02% (A), 0.05% (B), 0.1% (C), and 0.5% (D). Scale bars: 20 nm.

TEM Characterization of Au:Gel Nanoparticles in PBS at 0.2 wt% Gelatin

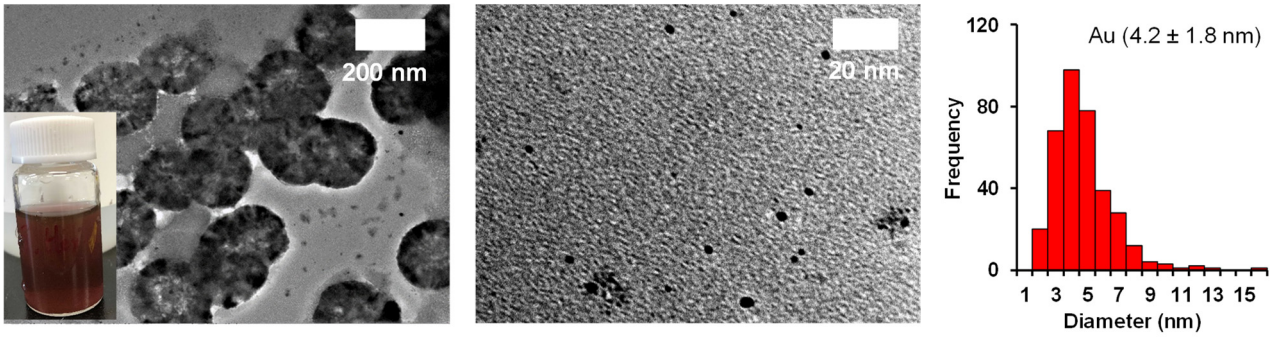


Figure S3. Transmission electron microscopy (TEM) images of Au:Gel nanoparticles at low (200 nm) and high (20 nm) magnification, along with corresponding particle size distribution histogram.

Circular Dichroism Spectra of Au:Gel Nanoparticles at Varying Gelatins

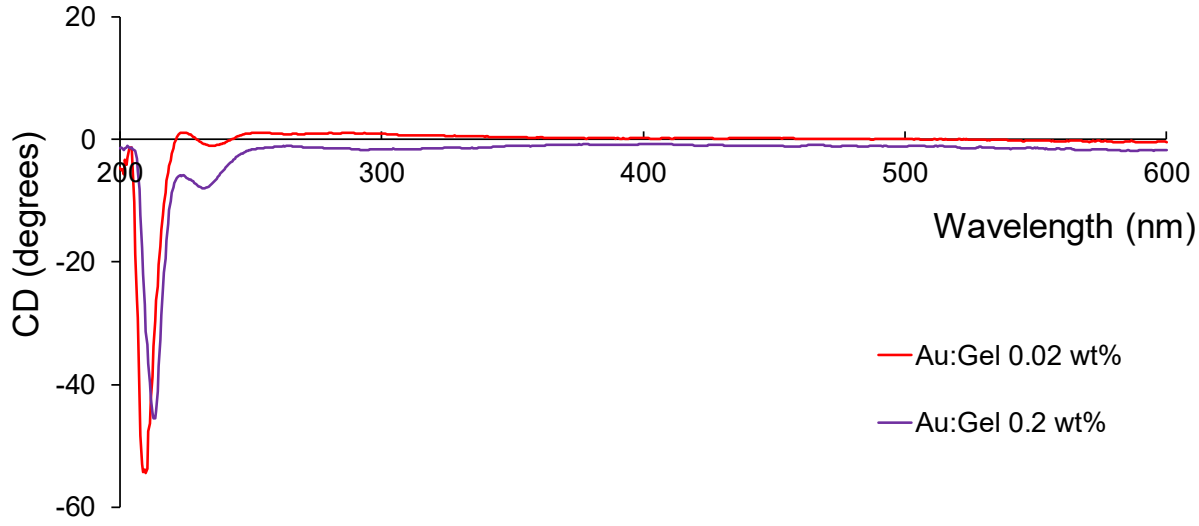


Figure S4. Circular dichroism (CD) spectra of Au:Gel nanoparticles at 0.02 wt% (red) and 0.2 wt% (purple) gelatin concentrations.

Zeta Potential Analysis of Gelatin and Au:Gel NPs

1 mL sample was placed into a disposable folded capillary cell (DTS1070). It was tightly capped. The bubbles were removed. The measurement (Nano-ZS, Malvern) was performed under 25 ^{0}C, with the number of measurements x 3, and a delay time of 60 s per measurement. All the measurements for each sample were repeated 3 times.

Table S1 Zeta potential measurements for Au:Gel (main experiment) and Gel (control experiment).

Loading of Gel,	**Zeta potential (mV)**	
wt %	***Gel***	***Au:Gel***
0.02	-17.3	-16.9
0.05	-21	-14.8
0.1	-11.3	-5.32
0.2	-12.2	-7.01
0.5	-14.2	-6.72